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SEA (ALPHA AMYLASE)

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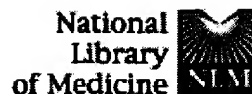
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FILE 'CAPLUS, BIOSIS, SCISEARCH, PASCAL, MEDLINE, CABA, FSTA, EMBASE,
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Improved thermostability of a Bacillus alpha-amylase by deletion of an arginine-glycine residue is caused by enhanced calcium binding.

Biochem Biophys Res Commun. 1998 Jul 20;248(2):372-7.

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Arginine is essential for the alpha-amylase inhibitory activity of the alpha-amylase/subtilisin inhibitor (BASI) from barley seeds.

Biochem J. 1993 Jul 1;293 (Pt 1):151-5.

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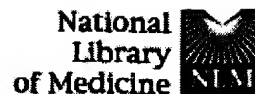
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Improved thermostability of a *Bacillus* alpha-amylase by deletion of an arginine-glycine residue is caused by enhanced calcium binding.

Igarashi K, Hatada Y, Ikawa K, Araki H, Ozawa T, Kobayashi T, Ozaki K, Ito S.

Tochigi Research Laboratories of Kao Corporation, 2606 Akabane, Ichikai, Haga, Tochi 321-3497, Japan.

alpha-Amylase from alkaliphilic *Bacillus* KSM-1378 (LAMY) is a novel semi-alkaline enzyme which has a high specific activity, a value 5-fold higher than that of a *Bacillus* licheniformis enzyme at alkaline pH. Thermostability of this enzyme could be improved deletion of the Arg181-Gly182 residue by means of site-directed mutagenesis. The wild-type and engineered LAMs were very similar with respect to specific activity, pH-activity curve, temperature-activity curve, susceptibility to inhibitors, and pattern of hydrolysis products from soluble starch and maltooligosaccharides. However, the engineered enzyme also acquired increased pH stability and resistance to sodium dodecyl sulfate and especially chelating reagents, such as ethylenediaminetetraacetate and ethyleneglycol-bis (beta-aminoethylether)tetraacetate. This is the first report that thermostability of alpha-amylase improved by enhanced calcium binding to the enzyme molecule. Copyright 1998 Academic Press.

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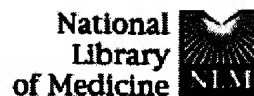
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Abe J, Sidenius U, Svensson B.

Department of Chemistry, Carlsberg Laboratory, Copenhagen Valby, Denmark.

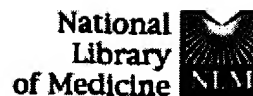
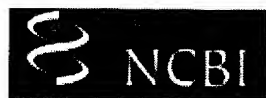
Treatment of barley alpha-amylase/subtilisin inhibitor (BASI) with reagents specific for arginine, histidine, methionine and tyrosine residues and amino and carboxyl groups indicates that an arginine residue(s) is essential for its action on the target enzyme barley alpha-amylase 2. Phenylglyoxal modified eight out of 12 arginine residues in BASI. Kinetic analysis shows that the inactivation of BASI follows a pseudo-first-order reaction and is due to reaction with one molecule of phenylglyoxal; the second-order rate constant determined to be $2.95 \text{ M}^{-1} \cdot \text{min}^{-1}$. At pH 8.0, BASI and barley alpha-amylase 2 form an inactive 1:1 complex. The K_i value of this association is $2.2 \times 10^{-10} \text{ M}$. The alpha-amylase protects four arginine residues and also the alpha-amylase inhibitory activity of BASI against phenylglyoxal. When BASI from the phenylglyoxal-modified target enzyme inhibitor complex is isolated and subjected to a second treatment with phenylglyoxal, for additional arginine residues are modified, with concomitant loss of the inhibitory activity. These results are discussed in relation to a three-dimensional model of BASI based on the known structure of the corresponding inhibitor from wheat.

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